Bacterial Transformation

What is transformation?

In molecular biology, transformation is the genetic alteration of a cell resulting from the **direct uptake and incorporation of exogenous genetic material** from its surroundings through the cell membrane(s).

State of Competence

- For transformation to take place, the recipient bacteria must be in a **state of competence**.
- **Competence** refers to a **temporary state of being able to take up exogenous DNA** from the environment.
- It occurs in **nature as a time-limited response** to environmental conditions such as starvation and cell density.
- It may be induced in a **laboratory**.

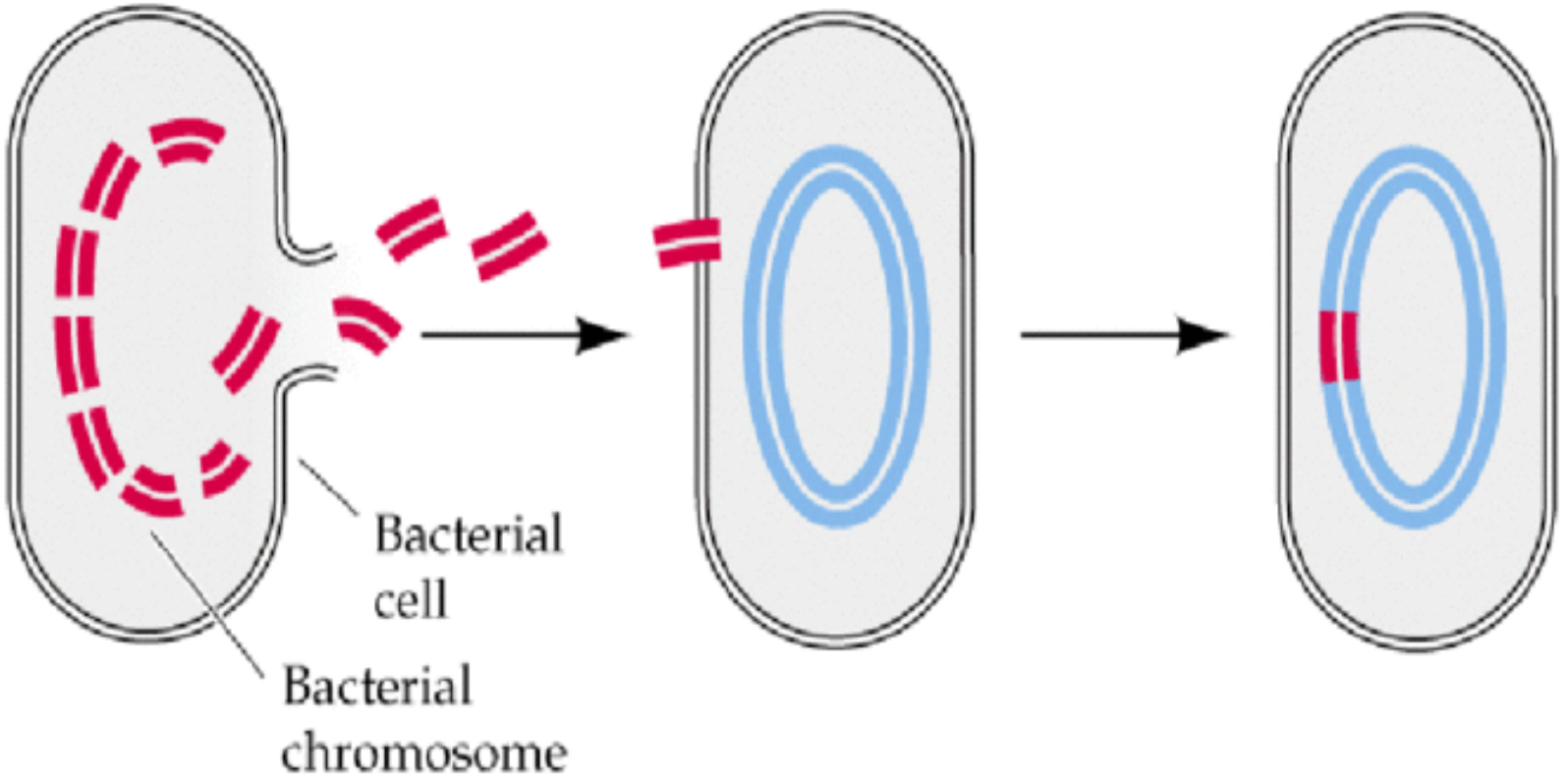
Transformation in nature

- In transformation, the genetic material passes through the intervening medium, and **uptake is completely dependent on the recipient bacterium.**

- Natural genetic transformation appears to be an **adaptation for repair of DNA damage** that also generates genetic diversity.

- Nearly **100 species of bacteria** are known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria.

Transformation



Transfection

- "Transformation" may also be used to describe the **insertion of new genetic material into nonbacterial cells**, including animal and plant cells.

- However, because "transformation" has a special meaning in relation to **animal cells**, indicating progression to a **cancerous** state, the process is usually called **"transfection".**

History

- Transformation in bacteria was first demonstrated in 1928 by the British bacteriologist **Frederick Griffith**.

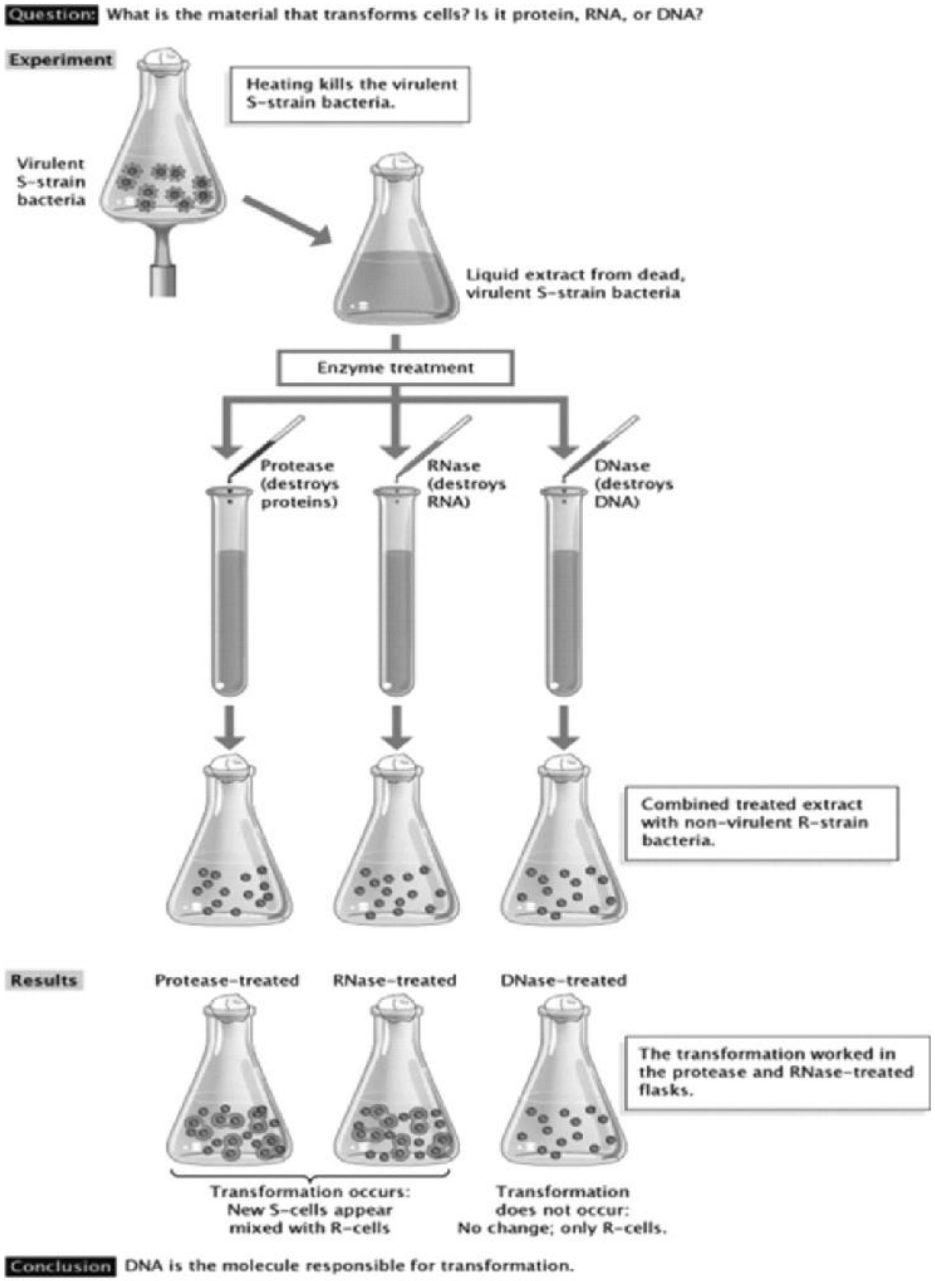
- He discovered that a **non-virulent strain** of *Streptococcus pneumoniae* could be **made virulent** after being exposed to heat-killed virulent strains.

- Griffith hypothesized that some **"transforming principle"** from the heat-killed strain was responsible for making the harmless strain virulent.

- In **1944** this "transforming principle" was identified **as being genetic** by **Oswald Avery** and **co-workers.**

- They **isolated DNA** from a virulent strain of *S. pneumoniae* and using just this DNA were able to make a harmless strain virulent.

- They named this uptake and incorporation of DNA by bacteria as **"transformation."**


Question: What is the material that transforms cells? Is it protein, RNA, or DNA?
Experiment
Heating kills the virulent S-strain bacteria.
Virulent S-strain bacteria
Liquid extract from dead, virulent S-strain bacteria
Enzyme treatment
Protease (destroys proteins)
RNase (destroys RNA)
DNase (destroys DNA)
Combined treated extract with non-virulent R-strain bacteria.
Results
Protease-treated
RNase-treated
DNase-treated
The transformation worked in the protease and RNase-treated flasks.
Transformation occurs: New S-cells appear mixed with R-cells
Transformation does not occur: No change; only R-cells.
Conclusion DNA is the molecule responsible for transformation.

- It was originally thought that *Escherichia coli*, a commonly used laboratory organism, cannot be transformed.

- However, in 1970, it was shown that *E. coli* may be induced to take up DNA from **bacteriophage λ.**

- Two years later in 1972 it was shown that $CaCl_2$ treatment is also effective for transformation of **plasmid DNA.**

Natural competence and transformation

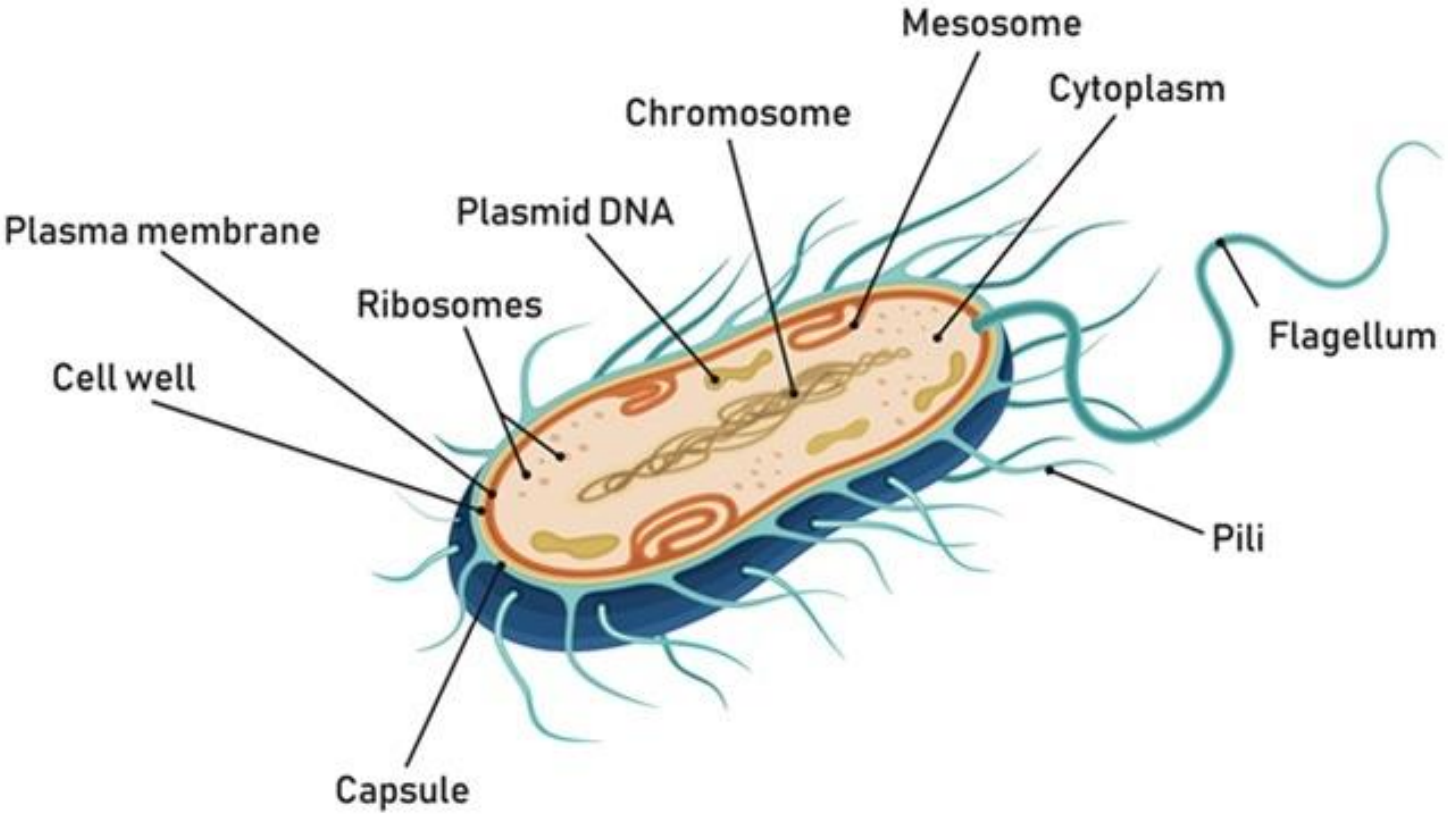
- Naturally competent bacteria carry **sets of genes** that provide the **protein machinery** to bring DNA across the cell membrane(s).

- The transport of the exogenous DNA into the cells may require proteins that are involved in the assembly of **type IV pili** and **type II secretion system**, as well as **DNA translocase complex** at the cytoplasmic membrane.

- type IV pili are used by bacteria for locomotion

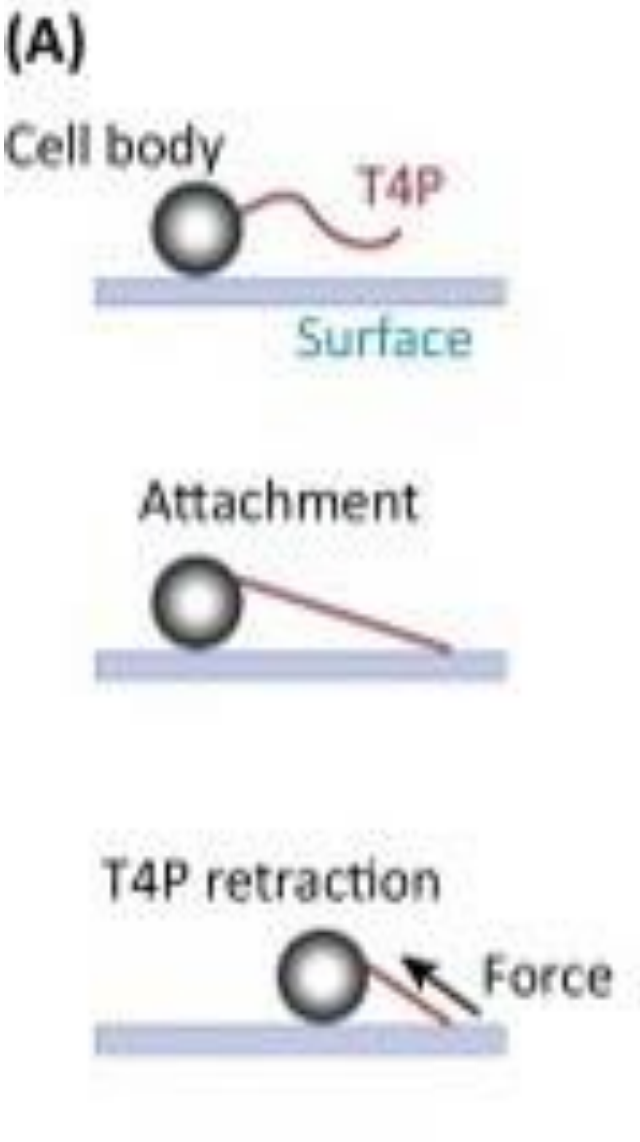
- The type II secretion system is a membrane bound protein complex found in Gram-negative bacteria that is used to secrete proteins found in the cytoplasm of the bacteria into the extracellular space outside of the cell.

STRUCTURE OF A BACTERIAL CELL



Role of Type IV pili

- **Type IV pili (T4P),** generate **motile forces**.
- The **external ends** of the pili adhere to a solid substrate, either the surface to which the bacterium is attached or to other bacteria.
- Then, when the **pili contract**, they pull the bacterium forward.
- Movement produced by type IV pili is typically jerky, so it is called **twitching motility**, as opposed to other forms of bacterial motility such as that produced by flagella.


(A)
Cell body
T4P
Surface
Attachment
T4P retraction
Force

DNA uptake

- Due to the **differences in structure** of the cell envelope between Gram-positive and Gram-negative bacteria, there are **some differences in the mechanisms of DNA uptake** in these cells.

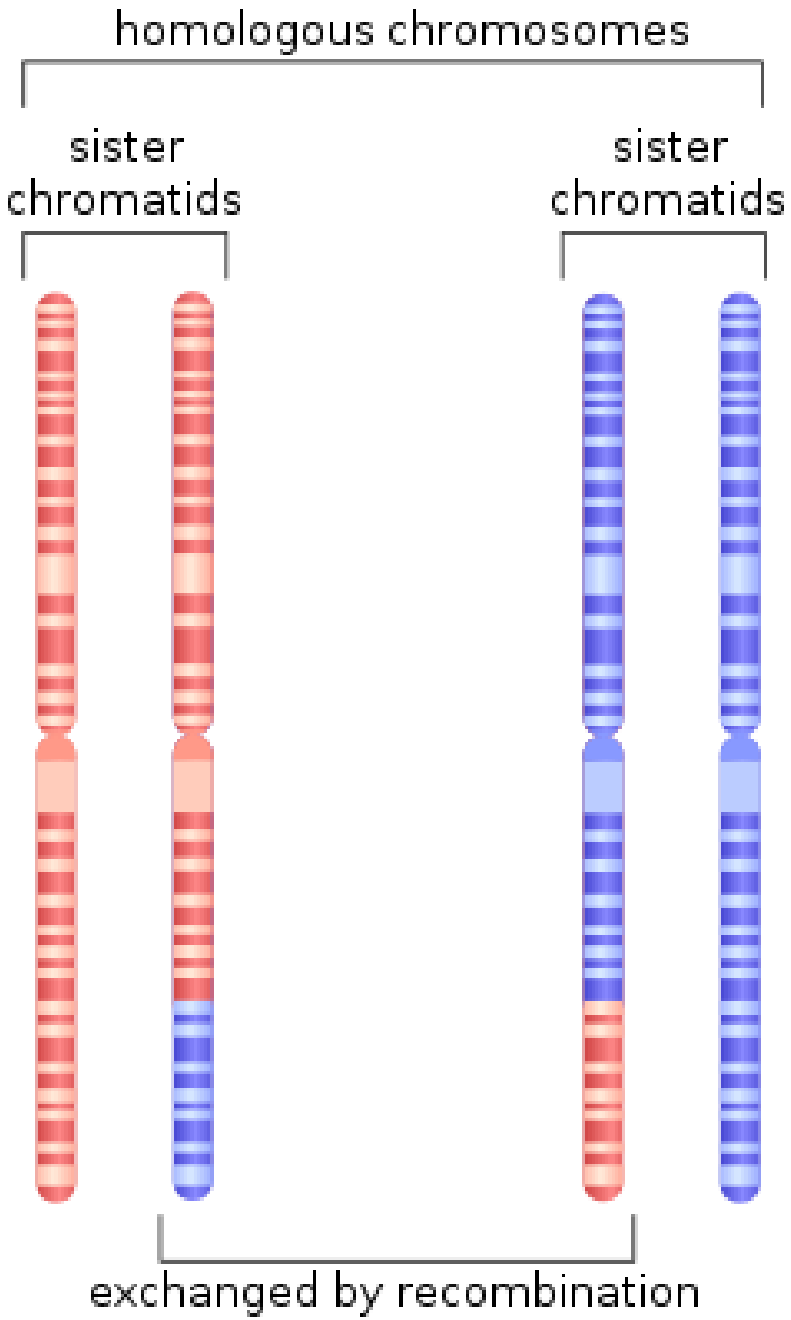
- However most of them **share common features** that involve **related proteins**.

- The DNA first binds to the surface of the competent cells on a **DNA receptor**, and passes through the cytoplasmic membrane via **DNA translocase**.

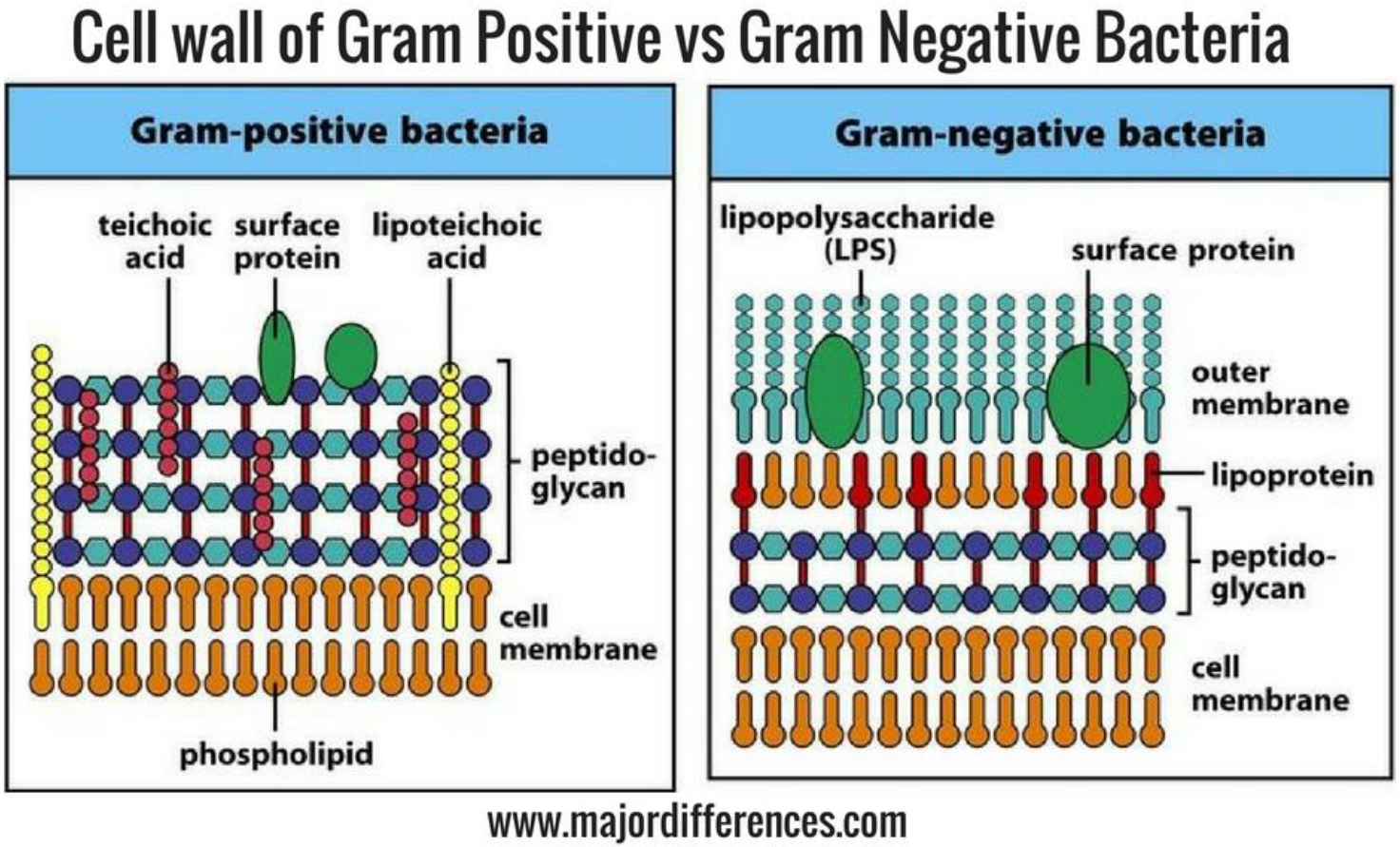
- Only **single-stranded DNA may pass through**, the **other strand being degraded** by nucleases in the process.

- The **translocated single-stranded DNA** may then be integrated into the bacterial chromosomes by a **RecA-dependent process**.

- **RecA** is an adenosine triphosphate (ATP)-dependent **DNA repair protein** that catalyzes the **DNA strand exchange reaction** in **homologous recombination**.


homologous chromosomes
sister chromatids
sister chromatids
exchanged by recombination

- In Gram-negative cells, due to the presence of an **extra membrane**, the DNA requires the presence of a channel formed by **secretins** on the outer membrane.



- **Pilin** may be required for **competence**, but its role is uncertain.

- The uptake of DNA is generally **non-sequence specific**, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

Transformation is a complex process

- In general, transformation is a **complex, energy-requiring developmental process.**

- As already mentioned, in order for a bacterium to bind, take up and recombine exogenous DNA into its chromosome, it **must become competent**, that is, enter a special physiological state.

- Competence development requires expression of many genes, e.g., in *Bacillus subtilis* expression of about **40 genes** is needed**.**

- The DNA integrated into the host chromosome is usually (but with rare exceptions) derived from another **bacterium of the same species**, and is thus **homologous** to the resident chromosome.

- In *B. subtilis* the length of the transferred DNA is greater than 1271 kb (more than **1 million bases**).

- This length is **more than a third of the total chromosome length** of 4215 kb.

- It appears that about **7-9% of the recipient cells take up an entire chromosome.**

- The capacity for natural transformation appears to occur in a number of prokaryotes, and thus far **67 prokaryotic species** (in seven different phyla) are known to undergo this process.

Conditions for transformation

- **Competence** for transformation is typically **induced by high cell density and/or nutritional limitation**, conditions associated with the **stationary phase** of bacterial growth.

- Transformation in *Haemophilus influenzae* occurs most efficiently at the **end of exponential growth** as bacterial growth approaches stationary phase.

- Transformation in *Streptococcus mutans*, as well as in many other streptococci, occurs at **high cell density** and is **associated with biofilm formation.**

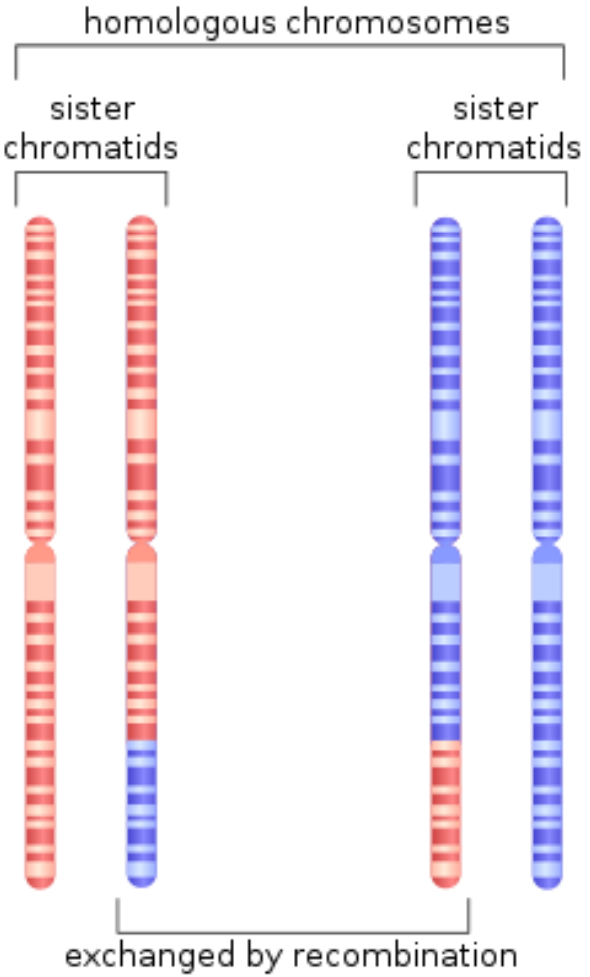
Need for transformation

- Transformation is an **adaptation for DNA repair**.
- Competence is **specifically induced by DNA damaging conditions.**
- For instance, transformation is induced in *Streptococcus pneumoniae* by the DNA damaging agents **mitomycin C** and **fluoroquinolone**.
- In *B. subtilis*, transformation is increased by **UV light, a DNA damaging agent.**

Homologous recombinational repair (HRR)

- The process of **homologous recombinational repair (HRR)** is a key DNA repair process that is especially effective for **repairing double-strand damages**, such as double-strand breaks.

- This process depends on exchange of DNA from a **second homologous chromosome to the damaged chromosome.**



Effect of stage of growth

- Logarithmically growing bacteria **differ** from stationary phase bacteria with respect to the **number of genome copies** present in the cell.

- This has implications for the **capability** to carry out an important DNA repair process.

- During **logarithmic growth**, **two or more copies** of any particular region of the chromosome may be present in a bacterial cell, as cell division is **not precisely matched** with chromosome replication.

- During **logarithmic growth**, a DNA damage in one chromosome may be repaired by HRR using sequence information from the **other homologous chromosome**.

- Once cells approach **stationary phase**, however, they typically have **just one copy of the chromosome**, and HRR requires **input of homologous template from outside the cell by transformation.**

Evolutionary importance

- Transformation in bacteria can be viewed as a **primitive sexual process**, since it involves **interaction of homologous DNA from two individuals** to form **recombinant DNA that is passed on to succeeding generations.**

- **Bacterial transformation** may have been the **ancestral process that gave rise to meiotic sexual reproduction in eukaryotes.**


Thank You!